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# Successful Suppression of the Early Rejection of Pig Islets in Monkeys

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Primary nonfunction (PNF) is seen very frequently after xenogeneic transplantation of islets of Langerhans. In a pig-to-rat model we recently observed that no PNF occurs when the islets are kept in culture at 37°C for 1–2 weeks prior to transplantation. In order to investigate the rejection mechanisms in a preclinical model, we transplanted cultured porcine islets under the capsule of both kidneys in four cynomolgous monkeys. Islets were isolated from adult sows by means of digestion with Liberase in University of Wisconsin solution (UWS). The digest was purified by a density gradient of OptiPrep in UWS. Highly purified (>95%) islets were cultured 1–2 weeks in RPMI. All monkeys showed significant titers of preformed anti-pig antibodies. The immunosuppression of the monkeys consisted of cyclophosphamide (Cy) (2 days), cyclosporin A (CsA), and prednisolone. Anticipating a fast rejection we carried out nephrectomies at different time points within 2 weeks after transplantation. Following unilateral nephrectomy, well-preserved islets with no signs of rejection were observed between 3 and 7 days posttransplant. Later, between days 11 and 15 posttransplant, histology in the first three animals demonstrated no islets. In the fourth monkey histology on day 11 showed islets with excellent morphology and some small focal infiltrates. The highest CsA blood levels (around 1000 ng/ml) were found in animals with the best graft survival. We conclude that cultured porcine islets can be grafted without hyperacute rejection in monkeys with preformed anti-pig antibodies. In the presence of high levels of CsA only marginal signs of a cellular immune response were observed 11 days after transplantation.

Key words: Islet of Langerhans; Xenotransplantation; Pig; Monkey

## INTRODUCTION

Today type I diabetes is still a common but severe disease with a high morbidity despite optimal insulin treatment. Transplantation of a pancreas or islets of Langerhans is at present the only option to cure diabetes. Because of the worldwide and growing human donor organ shortage, a possible treatment could consist of transplantation of porcine islets. The use of porcine islets is hampered by a number of problems. Pig islets are difficult to isolate, are very fragile, and transplantation often results in primary nonfunction (PNF). We recently investigated whether culturing of islets of adult pigs could improve the results of xenotransplantation in various models. After transplantation in T-cell-deficient nude mice we found that cultured islets, but not freshly isolated islets, restored normoglycemia. Further, we observed that no PNF was encountered when cultured islets were grafted in immunocompetent rat recipients (unpublished results).

In this study, in order to assess possible rejection mechanisms in a preclinical model, we transplanted cultured porcine islets under the kidney capsule in cynomolgous monkeys.

Islets were isolated from pancreases of large sows, highly purified (>95% purity) in our novel OptiPrep-University of Wisconsin solution (UWS) gradient (8) and cultured 1–2 weeks at 37°C. Next, the cultured islets were grafted under the capsule of both kidneys of four cynomolgous monkeys, which received clophosphamide (Cy) (2 days), cyclosporin A (CsA), and prednisolone. Nephrectomies were carried out at different time points between 3 and 15 days posttransplant for histology.

## MATERIALS AND METHODS

### *Animals*

Experiments were performed, in accordance with the *Principles of Laboratory Animal Care* (NIH publication No. 85-23, revised 1985) and institutional guidelines, in four outbred cynomolgous monkeys (*Macaca fascicu-*

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*laris*; Biomedical Primate Research Centre, Rijswijk, The Netherlands) weighing 5–12 kg. The animals were maintained on a regular diet of monkey chow (Hope Farms, The Netherlands) supplemented with fresh fruit and vegetables and had free access to water. For surgery the animals were anesthetized using isoflurane.

*Islet Isolation and Culture*

Islets were isolated from pancreases obtained from slaughterhouse sows weighing 200–300 kg. Isolation was preformed by means of collagenase digestion with Liberase-PI (Roche Diagnostics, Almere, The Netherlands) dissolved in UWS. After incubation the gland was dispersed by shaking and sieving (400 μm) for approximately 30 min in UWS on ice. For purification a density gradient of OptiPrep (Nycomed, Oslo, Norway) in UWS was used (8,9). The yield was expressed in islet equivalents (IEs). Purity was consistently over 95%. The purified islets were cultured in a humidified ambient atmosphere in bacteriological petri dishes (10 cm in diameter; Falcon) with 10 ml RPMI-1640 medium (ICN Biomedicals, Zoetermeer, The Netherlands), containing 4.2 mM sodium bicarbonate, 20 mM HEPES, 2 mM L-glutamine, and supplemented with 10% adult heat-inactivated porcine serum (ICN), with a medium change at days 1 and 7 after isolation.

*Islet Transplantation*

The cultured islets were harvested, washed, and resuspended in RPMI-1640 medium without serum. Immediately before the transplant procedure aliquots of the islet suspension, each containing 1500–8000 IEs, were pelleted and aspirated in silicone tubing connected to a 100-μl Hamilton syringe. After exposure through a midline incision, both kidneys each received two grafts varying from 1500–8000 IEs by infusion in the subcapsular space. For immunosuppression the animals received Cy at 1 day before transplantation (40 mg/kg) and at 1 day posttransplantation (10 mg/kg), daily CsA starting 1 day before transplantation using either CsA in oily solution (Sandimmune, 100 mg/ml) administered IM or as a microemulsion preconcentrate (Neoral drinking solution, 100 mg/ml) starting with 25 mg/kg IM, then subsequently daily doses of 10 mg/kg IM, supplemented by daily oral doses of 100 mg/kg Neoral. The aim was to reach CsA trough levels of at least 600 ng/ml. If the CsA levels were over 1000 ng/ml, the CsA doses were reduced. Prednisolone was given at 1 mg/kg per day starting on the day of transplantation.

*Nephrectomies and Histology*

For histology a unilateral nephrectomy was performed between 3 and 7 days posttransplant; the other kidney was removed between 11 and 15 days posttransplant. After Bouin fixation and paraffin embedding, serial 5-μm sections were taken at 500-μm intervals and stained with aldehyde fuchsin and Halmi (specific for beta cells).

*Analytical Procedures*

CsA blood levels were determined using a radioimmunoassay after alcohol extraction (INCSTAR Corp., Stillwater, MN). Monkey anti-pig antibodies were determined with a complement-dependent hemolytic assay, using pig red cells as targets.

## RESULTS

Anticipating a fast rejection, nephrectomies were carried out at different time points within 2 weeks posttransplantation. On day 4, histology of the first monkey demonstrated well-preserved islets with no signs of rejection. On day 15, no islets were found and an inflammatory infiltrate was present consisting of mononuclear lymphocytes. The second monkey underwent a nephrectomy on days 11 and 14. In both kidneys no islets could be discovered and an infiltrate was seen. These animals had CsA levels ranging between 300 ng/ml (day 0) and 700 ng/ml (day 15) for the first monkey and between 900 ng/ml (day 0) and 400 ng/ml (around days 7–11) for the second monkey. On day 7 a kidney of the third monkey showed an excellent preserved graft and no infiltrating cells. On days 3 and 11 histology of the fourth monkey also showed islets with excellent morphology while on day 11 some small focal infiltrates were seen consisting mainly of monocytes (Fig. 1). The highest CsA blood levels (around 1000 ng/ml) were found in animals showing graft survival at days 7 and 11. These results are summarized in Table 1.

Hemolytic anti-pig antibodies were determined before and after transplantation and were compared with a sample of human serum. The human serum sample had a titer of 1/20 in all assays performed. Anti-pig antibody levels were always present before and after transplantation in all four monkeys. Anti-pig antibodies increased from 1/20 (before transplantation) to 1/160 on day 14 in monkey 1; from 1/40 (before transplantation) to 1/80 (day 7 and 14) in monkey 2; remained 1/40 before transplantation and on day 7 in monkey 3; increased from 1/10 (before transplantation) to 1/20 (day 4 to 11) in monkey 4. Thus, only in the animal with the shortest follow-up and the highest CsA level could no significant increase in anti-pig antibody titer be observed.

## DISCUSSION

The use of porcine donor material could alleviate the need for human organs. Unfortunately, xenotransplanta-

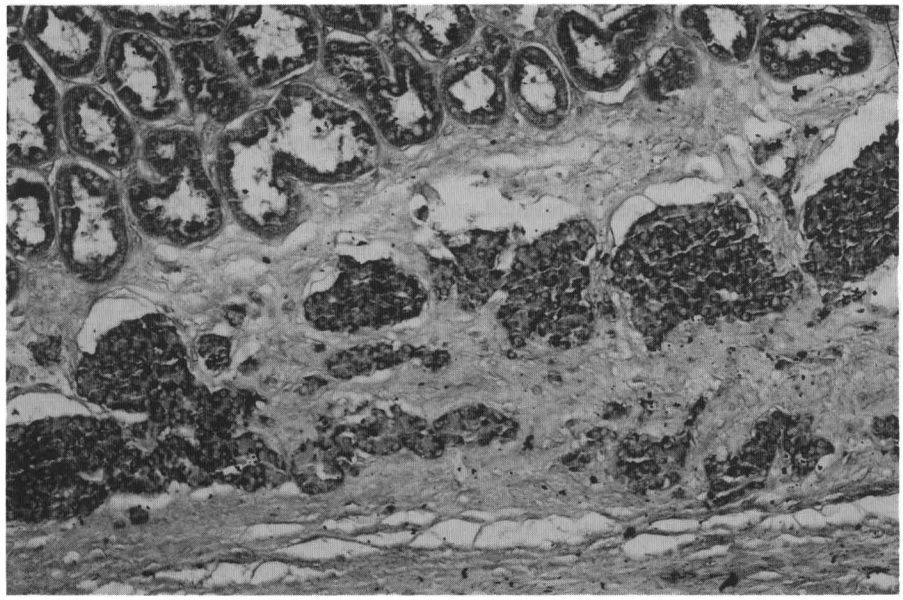

**Figure 1.** Photomicrograph of 1–2-week cultured adult porcine islets, transplanted under the renal capsule of a cynomolgous monkey. Immunosuppression consisted of cyclosporin A, cyclophosphamide, and prednisolone. Sections were stained with beta cell-specific aldehyde-fuchsin and Halmi. Islets with excellent morphology were seen on day 11 in the fourth monkey. Original magnification: ×100.

tion of porcine whole organs to primates invariably results in hyperacute rejection (HAR) unless these organs originate from pigs transgenic for human regulators of complement activation such as hDAF (10). It is still unclear whether this also applies to unvascularized grafts such as islets of Langerhans. In this study, we have demonstrated that no histological signs of HAR in any of the analyzed grafts could be found. Similar immunosuppression did not prevent HAR in pig-to-cynomolgous monkey heart transplants from nontransgenic pigs (9). This shows clearly in a preclinical model that cultured pig islets are not rejected in a hyperacute fashion by the preexisting anti-pig antibodies.

In the literature only a few studies have addressed this question in similar models. In most experiments islets of fetal pigs were used (3,4,7) and in these studies no indications for HAR were found. In one study (7) no deposits of IgM, IgG, or complement factors could be detected. Whereas whole organ grafts from DAF-transgenic donors demonstrate complete abrogation from HAR, no additional effect was observed with islet grafts from such donors (4). In a recent study using porcine

**Table 1.** Histology of Islet Graft Survival and CsA Through Levels

| | Monkey 1 | | Monkey 2 | | Monkey 3 | | Monkey 4 | |
|---|---|---|---|---|---|---|---|---|
| Day | Islet Histology | CsA Level (ng/ml) | Islet Histology | CsA Level (ng/ml) | Islet Histology | CsA Level (ng/ml) | Islet Histology | CsA Level (ng/ml) |
| 0 | | 324 | | 900 | | >1000 | | 685 |
| 3 | | | | | | | ++ | |
| 4 | ++ | 377 | | 896 | | >1200 | | 528 |
| 7 | | 334 | | 411 | ++ | >1000 | | 919 |
| 11 | | 611 | – | 463 | | | ± | >1000 |
| 14 | | | – | 718 | | | | |
| 15 | – | 695 | | | | | | |

++: islets present, no infiltrate; ±: islets present, infiltrate present; –: no islets present, inflammatory infiltrate present.

islets of unspecified donor age it was found that cultured islets reversed diabetes in five out of six diabetic cynomolgous monkeys (5). Mirenda et al. (6) studied adult porcine islets in relation to human preformed antibodies and found no harmful effects after in vitro incubation.

Because most of the above-mentioned studies used cultured islets, it is still unclear whether freshly isolated islets would be more vulnerable for antibody-mediated destruction. In a single study reporting transplantation of freshly isolated rabbit islets in the monkey, destruction within 6 h was observed (2). Taken together these results suggest that PNF caused by HAR is not a major problem in a pig-to-primate model. Our results further indicate that suppression of the early cellular xenograft rejection is achievable with a combination of immunosuppressive drugs, which is in line with other studies (1,4,7,10,11). It is still an open question whether these rigorous induction therapies can be reduced to clinically acceptable maintenance schedules.

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